PB80-177843

Use of the Grass Shrimp 'Palaemonetes pugio' in a Life-Cycle Toxicity Test

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1979

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECI
EPA-600/J-79-061	JOURNAL ARTICLE	PB80-177843

4. TITLE AND SUBTITLE
USE OF THE GRASS SHRIMP (PALAEMONETES PUGIO) IN A LIFE-CYCLE TOXICITY TEST

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Dana Beth Tyler-Schroeder

8. PERFORMING ORGANIZATION REPORT NO

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Same as Box # 12

10. PROGRAM ELEMENT NO.
1EA615

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory, Gulf Breeze
Office of Research and Development
U. S. Environmental Protection Agency, Sabine Island
Gulf Breeze, Florida 32561

13. TYPE OF REPORT AND PERIOD COVERED
final

14. SPONSORING AGENCY CODE
EPA/600/04

15. SUPPLEMENTARY NOTES
REFERENCE: Aquatic Toxicology, ASTM STP 667, L. L. Marking and R. A. Kimerle, Eds American Society for Testing and Materials, 1979, pp. 159-170

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The juveniles reached sexual maturity during the first two weeks of the exposure. Thereafter, a photoperiod regime was used to induce reproduction, and the effects of endrin on the survival, growth, and reproduction of the parent generation were determined. Larvae spawned by control and exposed parents were continuously exposed until the juvenile stage (7 to 20 mm in rostrum-telson length). The effects on survival, length of larval development, and growth of the F_1 generation were determined.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Aquatic animals Chemical properties insecticides	estuarine grass shrimp toxicity tests chlorinated hydrocarbon pesticide	06/F 08/A

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
release to public	unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	

Authorized Reprint from
Special Technical Publication 667
Copyright
American Society for Testing and Materials
1916 Race Street, Philadelphia, Pa. 19103
1979

JOURNAL ARTICLE
EPA-600/J-79-061

D. B. Tyler-Schroeder[1]

Use of the Grass Shrimp (*Palaemonetes pugio*) in a Life-Cycle Toxicity Test

REFERENCE: Tyler-Schroeder, D. B., **"Use of the Grass Shrimp (*Palaemonetes pugio*) in a Life-Cycle Toxicity Test,"** *Aquatic Toxicology, ASTM STP 667*, for L. L. Marking and R. A. Kimerle, Eds., American Society for Testing and Materials, 1979, pp. 159–170.

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The 96-h LC_{50} for juvenile grass shrimp was 0.35 μg endrin/litre water. In the life-cycle toxicity test, gonadal development and spawning were inhibited at 0.03 μg endrin/litre water. Because this was the lowest concentration tested, the maximum allowable toxicant concentration could not be determined. The specific application factor for grass shrimp is, therefore, less than 0.08, approximately one order of magnitude lower than those generated in life-cycle tests exposing freshwater and estuarine fishes to endrin.

KEY WORDS: toxicology, toxicity tests, endrin, life cycles, application factor, aquatic toxicology

Marine aquatic toxicologists have demonstrated that crustaceans, such as the penaeid and palaemonid shrimp, are often more sensitive to organic

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pollutants than many other marine and estuarine organisms [*1–4*].[2] Hazard assessment of pollutants must examine the pollutant's effects on a species throughout its entire life cycle, most importantly the reproductive stages, and on the F_1 generation. Few toxicity studies have been conducted with larval stages of shrimp, and laboratory attempts to induce reproduction have generally been unproductive. This paper reports the successful use of the grass shrimp (*Palaemonetes pugio*, Holthuis) in a life-cycle toxicity test with the chlorinated hydrocarbon pesticide endrin.

Life History

Palaemonetes pugio occur in estuaries and embayments along the Gulf and Atlantic Coasts of the Americas [*5,6*]. These animals prefer shallow, densely vegetated areas, where they play a major role in the breakdown of plant material and the transfer of energy [*7,8*]. Grass shrimp are a valuable food for predaceous estuarine fishes and birds [*9–14*]. They prefer a salinity range of 1 to 15 parts per thousand, but tolerate salinities up to 30 parts per thousand [*14*].

Egg production occurs most of the year (as early as March and until October or November, depending on the geographic location and temperature). The eggs hatch as planktonic zoeal larvae after two to three weeks of embryonic development while attached to the pleopods of the female [*14*]. The optimum salinity-temperature regime for larval development is 25 parts per thousand and 25°C [*15*]. The larval development is completed in 12 to 35 days by metamorphosis to the postlarval stage. The juvenile growth is rapid, and sexual maturity is usually attained within one to two months, depending on temperature. Females often deposit a new egg mass within one to two days after the hatching of the previous brood, the number of eggs correlating with the length of the female. Grass shrimp normally live one to two years [*14*].

Preliminary Studies

Laboratory-induced spawning of *P. pugio* was reported by Little [*16*], who used the stimuli of photoperiod and temperature, singly and in combination. Egg deposition occurred after five to eight weeks of conditioning. These techniques were redefined and adapted to life-cycle toxicity tests. The following information was obtained: (1) Spawning can be induced when the temperature is held constant at 25°C while the photoperiods increase from 10 to 12 h or longer. A constant temperature throughout a toxicity test eliminates possible temperature/toxicant response interactions. (2) Spawning may be induced two to three weeks after initiation of the temperature/photoperiod regime. (3) One-hundred-watt, 1750-lumen

[2]The italic numbers in brackets refer to the list of references appended to this paper.

incandescent light bulbs can be used instead of fluorescent or growth light bulbs to initiate spawning. (The latter types stimulate undesirable algal growth in the exposure tanks.) (4) Less than 10 h of light per day from 15-watt, 125-lumen incandescent bulbs will check or retard ovarian growth and egg deposition. Thus, sufficient exposure time should be allowed for uptake of the chemical pollutant by the shrimp before spawning is initiated.

Materials and Methods

Test Animals

Grass shrimp were collected from a natural population near the Environmental Protection Agency's Environmental Research Laboratory in Gulf Breeze, Fla. The animals were acclimated in the laboratory for ten days prior to exposure. Acclimation was conducted in exposure aquaria under conditions of relative darkness to inhibit premature ovarian growth and egg deposition. The test animals and their foods were chemically analyzed and found to be free of significant levels of chlorinated hydrocarbons.

Test Water

The acclimation and test water was pumped from Santa Rosa Sound, filtered (20 μm), and heated to 25 $\pm$ 2°C. The salinity of the test water for the entire exposure averaged 20 parts per thousand (with a range of 12 to 28 parts per thousand).

Exposure Apparatus

All exposures were conducted in a flow-through system, using a modified Mount and Brungs [*17*] diluter to deliver one half of a litre of exposure water to each tank per cycle, with a dilution ratio of 50 percent. The flow rate was adequate to ensure 95 percent replacement in exposure aquaria in 24 h [*18*].

Chlorinated hydrocarbon pesticides, such as endrin, are insoluble in seawater. In toxicity testing, these materials must be solubilized by adding a carrier (acetone, triethylene glycol, ethanol, or others) to the exposure medium. The carrier in these experiments was triethylene glycol (TEG). For uniformity, the diluter was modified to maintain an equal carrier concentration in all the toxicant concentrations and the carrier-control water [*19*]. The toxicant and carrier were delivered to the diluter by a Lambda[3] pumping system.

[3]The Lambda pumps were manufactured by the Harvard Apparatus Co., Inc., Millis, Mass. Reference to commercial products does not constitute endorsement by the Environmental Protection Agency.

Many diluter systems normally prepare equal volumes of toxicant and carrier-control exposure media for each cycle. However, the diluter utilizes only approximately one half as much toxicant water per cycle as carrier-control water; the excess toxicant water overflows to a drain. Our diluter was modified to allow the preparation of unequal volumes of toxicant and carrier-control water for each cycle, which prevented the production of excessive toxicant water.

A pulse multiplier, designed by Rauf Argon, Inc.,[4] allowed the delivery of unequal volumes of toxicant and carrier stocks to the respective mixing boxes, which resulted in identical carrier concentrations for both boxes. The electrical impulse driving the pump delivering the endrin stock was multiplied and used to drive a second pump delivering carrier to the carrier-control mixing box. The multiple chosen was the next higher whole number closest to the actual ratio between the volumes of the toxicant mixing box and the carrier-control mixing box.

For example, the volume of the toxicant mixing box in the exposure apparatus used in this study was 948 ml, whereas the carrier-control mixing box contained 2515 ml, a ratio of 1:2.5. The pulse multiplier, therefore, was set to deliver three times as much material (32 percent distilled water and 68 percent TEG). The concentrations of the carrier were equal in the carrier-control mixing box, the toxicant mixing box, and all the test aquaria: 0.0813 ml TEG/litre of exposure water.

Test Regime

Grass shrimp were exposed to endrin throughout an entire life cycle to assess the possible effects of the chemical on their survival, growth, reproduction, larval development, and metamorphosis. One hundred juvenile shrimp (12 to 19 mm in rostrum-telson length) were used per exposure aquarium. The shrimp were observed daily, and the dead animals were removed. Counts of the parental generation survival were made at two weeks, one month, and five months (the conclusion of the experiment).

In the first two weeks, the light regime for the exposure was 8 h light: 16 h dark, using 15-watt incandescent light bulbs. After two weeks, the light regime was increased to 10 h light:14 h dark, using 100-watt incandescent light bulbs. The light regime was increased further by a 47-min increment at two-week intervals until a regime of 13 h light:11 h dark per day was reached.

The effect of endrin on reproduction was assessed for each test concentration by counting the number of females that spawned each day, the number of eggs produced per female, and the number of larvae hatched per female. Each day, ovigerous shrimp were separated from those that

[4]Rauf S. Argon, Bedford, Mass.

had not yet spawned and placed in a compartment separated from the rest of the aquarium by a 2000-μm nylon mesh screen partition.

The effect of endrin on hatching success was determined by placing ten females from each test concentration into a hatching apparatus (Fig. 1). The hatching apparatus for each test concentration consisted of a 6.35-mm tubing manifold that fed exposure water to five small spawning chambers constructed of 30-mm glass tubing fitted on each end with neoprene stoppers and input and output tubing. One ovigerous female was placed in each chamber. The exposure water flowing from the diluter through the chamber flushed hatched larvae from each chamber into an egg cup constructed of nylon mesh screen (363 μm) 13 mm high fitted on a Petri dish top 100 mm in diameter. The larvae were counted to determine the hatch-

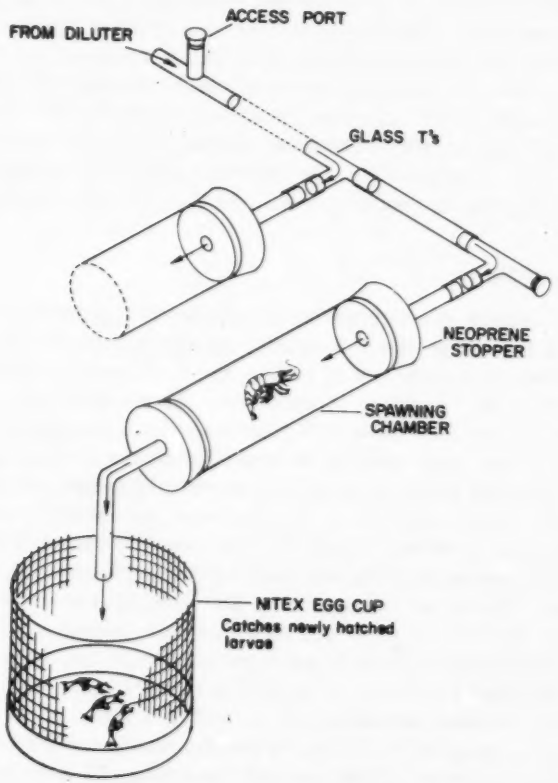

FIG. 1—*Apparatus utilized in a life-cycle exposure of grass shrimp to endrin to determine its effects on hatching success and larval viability. The access port was used for introducing* Artemia *nauplii to feed the ovigerous females in the spawning chambers.*

ing success per female shrimp and compared with egg counts for ten different females from each exposure concentration.

Larvae hatched from several females were pooled for exposure to the same concentration of endrin; a subsample was selected for the larval exposure portion of the experiment. The larvae developed in egg cups 150 mm in diameter and were fed freshly hatched *Artemia* nauplii daily. The exposure of these larvae in egg cups continued from the time of hatching through metamorphosis to the postlarval stage and through growth to the juvenile stage. A self-starting siphon in each larval exposure aquarium created fluctuating water levels in the tank and ensured an exchange of exposure medium in the egg cups.

The possible effect of endrin on growth was determined for the parental and F_1 generation shrimp. Thirty-five days after hatching, the rostrum-telson lengths of the F_1 shrimp that had metamorphosed to the postlarval stage were measured. The lengths and weights of all the parental and F_1 generation survivors were determined at the end of the test.

The whole-body concentrations of endrin in the shrimp were determined at the conclusion of the exposure. Because of differences in the reproductive history and length of exposure, residue analyses for each test concentration were performed on the parental shrimp that spawned, the parental shrimp that did not spawn, and the F_1 generation shrimp.

Supplemental Tests

The development of water quality criteria for the protection of aquatic life requires knowledge of acute toxicity, the bioconcentration potential of toxic materials, and estimates of "effect-no-effect" concentrations in life-cycle toxicity tests. Therefore, in addition to the life-cycle tests, the study determined (1) the acute 96-h LC_{50} (the concentration required to kill 50 percent of the test population in 96 hours) of endrin for first-day larvae, juveniles, and adult grass shrimp and (2) the rates of uptake and depuration of endrin in shrimp. Both determinations were conducted in continuous-flow systems, using syringe pumps to administer the endrin. Filtered seawater (20 μm) averaging 25°C was used for the larval, juvenile, and adult toxicity tests. The salinity averages were: for larvae, 12.4 parts per thousand (in a range of 12 to 14 parts per thousand); for juveniles, 23 parts per thousand (in a range of 22 to 24 parts per thousand); and for adults, 20.8 parts per thousand (in a range of 20 to 22 parts per thousand).

Unfiltered seawater averaging 25°C, and a salinity of 14 parts per thousand (in a range of 9 to 18 parts per thousand) was used in the bioconcentration exposure. Adult animals (averaging 23 mm in rostrum-telson length) were exposed to 0.1 μg of endrin/litre of water for 21 days. Thereafter, the shrimp were placed in endrin-free water to depurate for one week. Tissues were analyzed at three- to four-day intervals throughout the

exposure and daily during the depuration. The tissue samples (whole body shrimp) averaged 5.46 g and consisted of approximately 46 shrimp.

Statistical Analyses

The acute toxicity data were analyzed by linear regression after probit transformation to determine the 96-h LC_{50}s and the 95 percent confidence limits. The life-cycle test data were analyzed by standard analysis of variance techniques and appropriate multiple comparison methods for differences among means. Tests were performed to determine both the 95 and the 99 percent levels of confidence.

The data from the bioconcentration exposure were analyzed, using the nonlinear statistical uptake-depuration model developed by Bahner and Oglesby [*20*]. This model was used to calculate the bioconcentration factor (BCF) (the relationship between the concentration in the whole shrimp at wet weight and the concentration in the water) for the time period in which the chemical was in apparent equilibrium between the water and the shrimp tissues. The term "equilibrium" here denotes the period during which the concentration of endrin in shrimp tissue neither increases nor decreases. The author also calculated the time required to reach equilibrium and the time required for 90 percent depuration of endrin from shrimp tissues to take place.

Results

The data presented here are intended only to support a demonstration of the methods used in these experiments, not to be a definitive presentation of the results.

The ability of the grass shrimp (*Palaemonetes pugio*) to complete life-cycle functions was seriously impaired by exposure to concentrations of endrin significantly lower than the 96-h LC_{50} (Table 1). The 96-h LC_{50} for larval shrimp was 1.2 μg endrin/litre water; for juveniles, 0.35 μg/litre; and for adults, 0.69 μg/litre. Schimmel et al [*21*] reported a similar 96-h LC_{50} (0.63 μg/litre) for adult grass shrimp exposed to endrin. Its effects on reproduction were observed in the life-cycle toxicity test at all the exposure concentrations tested. Its effects on survival, larval development, and growth were observed at life-cycle test concentrations (Table 1) and are discussed below.

The survival of the parental generation was greatly affected after two weeks' exposure to endrin concentrations of 0.38 μg/litre and more (Table 2). At the test termination, the survival of parental shrimp exposed to concentrations of 0.11 μg/litre and more was less than that of the controls (Table 2).

The onset and duration of spawning were significantly delayed and

TABLE 1—*Toxicity of endrin to grass shrimp* (Palaemonetes pugio) *in a 145-day life-cycle exposure.*[a]

Generation	Life Stage or Function	Effect	Endrin Concentrations Producing the Effect, μg/litre
Parental	juvenile adult	death	0.38, 0.79
	reproduction	delay in onset of spawning	0.03, 0.05, 0.11, 0.18, 0.38, 0.79
		reduction in no. of females spawning	
F_1	larvae	death	0.11, 0.18, 0.38, 0.79
	postlarval stage	delayed metamorphosis	
		decrease in growth, final length	0.1, 0.18, 0.38, 0.74
		decrease in growth, final weight	0.05, 0.11, 0.18, 0.38, 0.79

[a]The mean measured concentrations of endrin were: 0 (controls), 0.03, 0.05, 0.11, 0.18, 0.38 and 0.79 μg/litre. The acute 96-h LC_{50} for juvenile grass shrimp was 0.35 μg endrin/litre.

TABLE 2—*Survival of parental generation shrimp in a 145-day life-cycle toxicity test with the chrlorinated hydrocarbon pesticide endrin.*[a]

Concentration	11/8/76	11/22/76	12/14/76	3/30/77
Seawater control	100	99	99	67
TEG control	100	99	99	65
0.03 μg/litre	100	100	99	41
0.05 μg/litre	100	100	99	64
0.11 μg/litre	100	99	96	40
0.18 μg/litre	100	95	92	51
0.38 μg/litre	100	61	58	26
0.79 μg/litre	100	8	6	...

[a]The 96-h LC_{50} for adult grass shrimp with endrin is 0.6 μg/litre.

lengthened for female grass shrimp at all exposure concentrations (Fig. 2). The number of females depositing eggs was also significantly smaller than that of the controls. Egg production and hatching success apparently were not affected by any of the concentrations tested.

The larval mortality, length of time to metamorphosis, and growth of juvenile shrimp were impaired by endrin concentrations of 0.11 μg/litre and higher. The final length (rostrum-telson) of juvenile shrimp exposed to concentrations of 0.11 μg endrin/litre of water and higher was 12 to 65 percent shorter than the final length of the control shrimp. The final

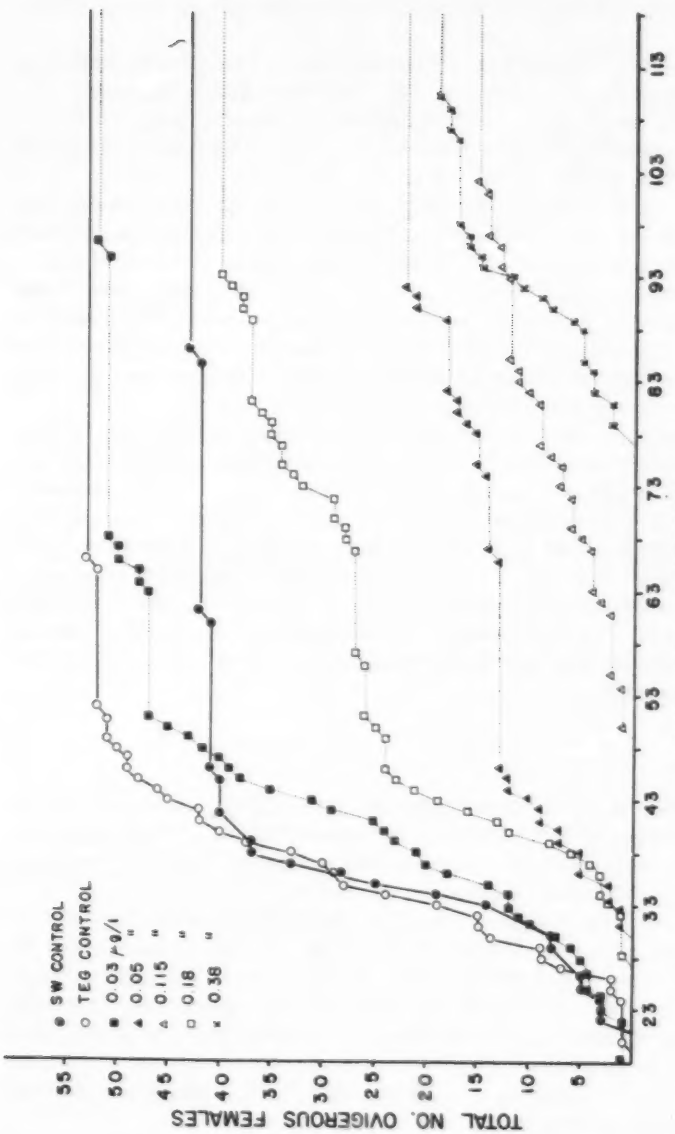

FIG. 2—*Effects of endrin on the reproductive success of grass shrimp exposed to several concentrations in a life-cycle toxicity test. The exposures began with 100 shrimp per test concentration; the natural sex ratio for* P. pugio *is 50 percent female, 50 percent male. SW = seawater; TEG = triethylene glycol.*

weight of juvenile shrimp exposed to concentrations of 0.05 μg endrin/litre of water and higher was 26 to 94 percent less than the final weight of the control shrimp. In contrast, the growth of parental shrimp was unaffected by endrin.

At the point of equilibrium, the concentration of endrin in shrimp tissue in the bioconcentration exposure was 1487 times that in the water. This equilibrium, or point of maximum uptake, was reached after 2.5 days of exposure to endrin. The time required for achieving 90 percent depuration of endrin from the tissues was 4.2 days.

The bioconcentration factor (BCF) for animals surviving the life-cycle toxicity test was calculated by a direct comparison of the amount of endrin in the shrimp tissue and that in the exposure water for each of the six concentrations tested. The average BCF so obtained (×1600) was similar to that obtained from the bioconcentration experiment. There were no apparent differences in the BCF of shrimp surviving the life-cycle test that were attributable to the concentration of pollutant in water, the spawning history, or the age of the shrimp.

The maximum allowable toxicant concentration (MATC) [*22*], a theoretically "safe" concentration of toxicant in water, falls somewhere between the "effect-no-effect" concentration for the most sensitive parameter examined in a life-cycle toxicity test. A "no-effect" concentration was not determinable from the results of these tests; therefore, the MATC for grass shrimp exposed to endrin could not be determined. The upper value of the range, however, was 0.03 μg endrin/litre of water. A specific application factor (MATC ÷ juvenile 96-h LC_{50}) [*22*] could not be calculated, but the author estimates that the factor should be less than 0.08 (0.03 μg/litre ÷ 0.35 μg/litre).

Discussion

The grass shrimp (*Palaemonetes pugio*) is useful in life-cycle toxicity tests. All stages of its life cycle can be exposed in flow-through apparatus in long or short-term tests. Spawning can be induced readily by increasing the photoperiod without altering the temperature.

At present, the opossum shrimp, or mysid, is the only other marine invertebrate utilized in life-cycle toxicity tests. The mysid has a short life cycle and direct development of the young, and is primarily a water-column organism. The grass shrimp lives one to two years, has an extended pelagic larval development, including a metamorphosis to a postlarval stage, and as an adult, is directly associated with detritus and sediments. The use of both organisms is complementary because they typify different life cycle types of estuarine and marine crustaceans.

Examination of the data from invertebrate life-cycle toxicity tests further demonstrates the need for toxicity tests that can (1) assess the effects on reproduction and (2) be used to determine MATCs. Because the specific

TABLE 3—*Comparison of the application factors generated for endrin using freshwater and estuarine fishes and an estuarine invertebrate.*[a]

Species	Habitat	Concentration, μg/litre		Application Factor	Reference
		96-h LC_{50}	MATC[b]		
Cyprinodon variegatus	saltwater	0.34	>0.12 <0.31	0.35	*24,25*
Jordanella floridae	freshwater	0.86	>0.22 <0.3	0.25	*24,25*
Palaemonetes pugio	saltwater	0.35	>? <0.08	<0.08	this paper

[a]The application factor equals the no-effect concentration divided by the 96-h LC_{50}.
[b]MATC = maximum allowable toxicant concentration.

application factor for grass shrimp with endrin is approximately an order of magnitude lower than the specific application factor for freshwater or saltwater fish (Table 3) [*24,25*], the need to test invertebrates to provide data for setting water quality criteria is obvious. This is particularly true for species like the grass shrimp because of their importance in the functioning of estuarine ecosystems, both as food for predators and in cycling detrital material into the food web.

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